REPORT FOR THE
INTERNATIONAL COOPERATION ON COSMETICS REGULATION

ICCR Working Group: Characterization of Nanomaterials II - Insolubility, Biopersistence and Size Measurement in Complex Media

Authors:
Hubert Rauscher, Ph.D.; Jay Ansell, Ph.D., DABT; Daisuke Araki, Ph.D.; Robert Bronaugh, Ph.D.; Yoshiaki Ikarashi; Marc-André Lefebvre, Ph.D.; Hiromichi Ogasawara, Ph.D.; Takahiko Suwa, D.M.V., Ph.D.; Azam F. Tayabali, Ph.D.; Ian Tooley, Ph.D.; Naoyuki Yasuda

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1. PURPOSE

To examine methods to characterize solubility, stability and persistence in biological media, and measurement of size in the realm of 1 to 100 nm in final formulations.

The International Cooperation on Cosmetics Regulation (ICCR) held its fifth annual meeting (ICCR-5) June 28-July 1, 2011 in Paris, France to discuss issues related to cosmetics and cosmetic-like drug/quasi-drug products[i].

Specific to nanotechnology, the meeting concluded:

- *Regulators accepted the report of the ICCR Ad Hoc Working Group on Characterization of Nanomaterials, which describes the characterization methods listed in the annex to the "Report of the ICCR Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: Criteria and Methods of Detection – ICCR-4."*

- *A new Working Group will be formed to examine methods to characterize insolubility, biopersistence, measurement of size in the realm of 1 to 100 nm in final formulations.*

- *Regulators and industry received an update on the progress of the Ad Hoc Working Group on Safety Assessment of Nanomaterials.*

Based on the 2011 ICCR-5 conclusion, a new Joint Working Group was formed. New Terms of Reference were endorsed by ICCR during the joint Industry/Regulators quarterly call of Feb 1, 2012, and the Working Group tasked with providing an analysis of strategies and methods to

[i] A more comprehensive discussion of the outcomes from this and previous meetings may be found at the European Commission's web site at:
http://ec.europa.eu/enterprise/sectors/cosmetics/cooperation-trade/international-level/
Health Canada's web site at:
http://www.hc-sc.gc.ca/cps-spc/person/cosmet/info-ind-prof/iccr-eng.php
Japan's Ministry Health, Labor and Welfare web site at:
http://www.mhlw.go.jp/bunya/iyakuhin/keshouhin/iccr03.html
and U.S. FDA's web site at:
http://www.fda.gov/InternationalPrograms/HarmonizationInitiatives/ucm114513.htm

characterize the criteria of solubility, stability, and persistence in biological media, and measurement of size in the realm of 1 to 100 nm in final formulations.

The Joint Working Group was charged with completing the report for review by ICCR at the ICCR-6 meeting, July 2012.

2. SCOPE

Examine the technical approaches and challenges to further define the criteria: solubility; stability and persistence in biological media; and measurement of size in the realm of 1 to 100 nm in final formulations.

Following the presentation of the report of the Ad Hoc Working Group on Characterization, "*Report of the Joint Regulator - Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials"* at the 2011 ICCR meeting, the Working Group noted that not all criteria from the 2010 ICCR-4 report were addressed in the 2011 ICCR-5 report. Therefore, the Working Group recommended a new project be undertaken to address the characterization of those additional criteria.

Specifically, the Working Group recommended developing a report that addresses methods and strategies that are useful to characterize:

- Solubility
- Stability and persistence of nanomaterials in biological media
- Measurement of size in the realm between 1 and 100 nm in the final formulation (i.e., in complex media)

As in the ICCR-5 Characterization report, the Working Group recognizes that there are a large number of methods referenced in the literature that might be used to characterize these parameters. These methods range from the experimental to those that can be considered mature and well developed. There are also numerous methods that could be applicable to the characterization of nanomaterials under very specialized conditions. However, these are

considered beyond the scope of this report, which will be limited to only the most relevant, well-developed methods for the specified parameters.

It is also important to acknowledge that the state of the science is constantly progressing. However, where analytical methods are not available as of the date of this report for precise quantification of each of the parameters, the Working Group will endeavor to identify specific challenges and, to the extent possible, identify possible approaches to the characterization of the identified criteria.

3. ACRONYMS AND DEFINITIONS

- ASTM — American Society for Testing and Materials
- AUC — Analytical Ultracentrifugation
- BET — Brunauer Emmett Teller method based on nitrogen adsorption
- CEN — European Committee for Standardization
- CHDF — Capillary Hydrodynamic Fractionation
- CPS — Centrifugal Particle Sedimentation
- DIN — Deutsches Institute für Normung (German Institute for Standardization)
- DLS — Dynamic Light Scattering
- DMEM — Dulbecco`s Modified Eagle Medium
- EDS — Energy Dispersive Spectroscopy
- EELS — Electron Energy Loss Spectroscopy
- FFF — Field Flow Fractionation
- ICCR — International Cooperation on Cosmetics Regulation
- ICP-MS — Inductively Coupled Plasma Mass Spectrometry
- ISO — International Organization for Standardization
- JRC — European Commission Joint Research Centre
- LD — Laser Diffraction
- NIST — United States National Institute of Standards and Technology
- OECD — Organisation for Economic Co-operation and Development
- PTA — Particle Tracking Analysis
- RPMI — Roswell Park Memorial Institute
- SAXS — Small Angel X-ray Scattering

- SEM Scanning Electron Microscopy
- SMPS Scanning Mobility Particle Size
- SOP Standard Operating Procedure
- SRM Standard Reference Nanomaterials
- TEM Transmission Electron Microscopy
- ToR Terms of Reference
- UV-VIS Ultraviolet–visible spectroscopy
- WDX Wavelength-Dispersive X-ray spectrometry
- WPMN Working Party on Manufactured Nanomaterials (OECD)
- XDC X-Ray Disc Centrifuge

4. Working Group Membership

1. Jay Ansell, Ph.D., DABT, Personal Care Products Council, U.S. (Co-Chair)
2. Daisuke Araki, Ph.D., Kanebo Cosmetic, Japan
3. Robert Bronaugh, Ph.D., Consultant, U.S. Food and Drug Administration, U.S.
4. Yoshiaki Ikarashi, National Institute of Health Sciences, Japan
5. Marc-André Lefebvre Ph.D., L'Oreal, France
6. Hiromichi Ogasawara, Ph.D., Office of OTC/Generic Drugs, Pharmaceuticals and Medical Devices Agency, Japan
7. Hubert Rauscher, Ph.D., European Commission - Joint Research Centre, Italy (Co-Chair)
8. Takahiko Suwa, D.V.M., PhD., Shiseido Co., Japan
9. Azam F. Tayabali, Ph.D., Health Canada, Canada
10. Ian Tooley, Ph.D., Croda International, England
11. Naoyuki Yasuda, Ministry of Health, Labour and Welfare, Japan

5. DISCUSSION

5.1. Introduction to Characterization of Nanomaterials in the International Cooperation of Cosmetics Regulations (ICCR) Initiative.

Nanotechnology has been an ongoing topic of discussion at ICCR since its inaugural meeting in 2007. At the 2nd annual meeting (ICCR-2) July 30-August 1, 2008, ICCR agreed that an ICCR Nanotechnology Working Group should be established. Based on this recommendation, a special

"International Workshop on Regulatory Issues Regarding the Use of Nanotechnology in Cosmetics" was convened in Ispra, Italy in July 2009. Hosted by the European Commission's Joint Research Centre, its purpose was to share the current approaches and knowledge on nanomaterials in cosmetics, and to more thoroughly explore the challenges of regulating them. (ISPRA 2009)

One of the two break-out sessions of this workshop, titled "Definition – Substance Identification, Detection and Characterization," concluded the following:

> "Overall the group agreed that a complete characterization, as would be needed for the scientific characterization of nanomaterials within a hazard identification and risk assessment framework, was far more detailed than that needed within a regulatory framework. It was agreed that for regulatory purposes simpler criteria, like those advanced within the ICCR framework would be sufficient. Even so, additional work would be needed to fully clarify terminology like stable, insoluble, or size" (1 to 100 nanometers?)."

The outcomes of the Ispra workshop were reported and discussed at the 3rd annual ICCR meeting (ICCR-3), September 2009 in Tokyo, Japan. While recognizing that a number of international authorities are working on technical definitions of nanomaterials, few were focusing on whether a particular material falls within the purview of any particular regulatory definition for nanomaterials, and none, at that time, addressed nanomaterials within the context of cosmetic products[ii]. Therefore, it was concluded that ICCR, with its focus on cosmetics, was in a strong position to establish a set of criteria that are consistent with international definitions but most relevant to cosmetics and could become the basis for criteria within the four regions.

[ii] As of the date of this report there are now a number of criteria documents that are intended to identify a nanomaterial for regulatory purposes. Of particular note, these include the 2009 definition for nanomaterials specifically in the context of cosmetic regulations published by the European Union (European Commission 2009). Others, like that of the U.S. FDA (FDA 2011), are broader but would encompass materials used in cosmetics. See Appendix 1 for selected examples.

In consideration of the above outcomes, ICCR regulators and industry representatives agreed to establish an Ad Hoc Working Group to identify and recommend a set of criteria to help determine whether a specific material used in cosmetics is to be considered a "nanomaterial" for regulatory purposes.

Those criteria were developed and the *Report of the ICCR Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: Criteria and Methods of Detection - ICCR-4* presented to the ICCR members at the 4th meeting (ICCR-4) held July 14, 2010 in Toronto, Canada.

> *Criteria*
> *For purposes of the International Cooperation on Cosmetic Regulation, a substance used in a cosmetic is considered a nano-ingredient if it is an insoluble ingredient, intentionally manufactured, with one or more dimensions in the realm of 1 to 100 nanometers in the final formulation and is sufficiently stable and persistent in biological media to allow for the potential of interaction with biological systems.*

Following a discussion of the report, ICCR established a new Ad Hoc Working Group that was directed to provide additional information on those methods identified in the 2010 ICCR-4 nanomaterial report. More specifically, the Working Group would direct itself to those parameters identified in Table 1, "Currently Available Methods for Characterization of Nanomaterials."

At the 5th annual meeting (ICCR-5) held June 29, 2011 in Paris, France, an introduction to the most relevant methods for the characterization of the parameters identified in Table 1 was presented.

In the 2011 report, experts noted that there were, in fact, criteria from the 2010 ICCR-4 report not addressed in Table 1, and thus not included in the ICCR-5 report. Therefore, the Working Group recommended a new project be undertaken to address the characterization of those additional criteria.

Specifically, the Working Group recommended developing a report that addresses methods that are useful to characterize the other properties identified in the 2010 Criteria Report. In particular:

- Solubility
- Stability and persistence of nanomaterials in biological media
- Measurement of size in the realm between 1 and 100 nm in the final formulation (i.e., in complex media)

5.1.1. Clarification of the Criteria for the Purposes of This Report

In order to facilitate understanding of the methodological discussion, the Working Group felt that it was important to clarify the meaning and purpose of the criteria addressed in this report.

5.1.1.1. **Criterion:** *Measurement of size in the realm between 1 and 100 nm in the final formulation (i.e., in complex media)*

5.1.1.1.1. "In the Realm of …"

While the scientific unit "nanometer" is quite precisely defined as a unit measure, its physiological significance is far from clear. Indeed, it should be emphasized that there is no established risk specifically attributed to nanotechnology, and size alone is not, in itself, an indicator of toxicity. There is no supporting evidence that provides for a bright line size limitation with respect to biological activity. Indeed, for mechanical systems, scaling laws are quite accurate on the nanoscale. This is not the case for electromagnetic properties where many scaling laws fail dramatically and predictions have shown variable accuracy in thermal systems. By example the National Nanotechnology Initiative (NNI), in its definition, uses the modifier "understanding and control of matter at dimensions of roughly 1 to 100 nanometers, where unique phenomena enable novel applications." Likewise, many of the definitions listed in Appendix 1 do not apply rigid size limits to the concept of the nanoscale. The 2010 ICCR Nano Criteria Working Group agreed and concluded the size should be made an approximation.

5.1.1.1.2. Stated Particle Size: Primary Particle, Aggregates or Agglomerates

The reproducibility and accuracy of any of the methods used for nanomaterial characterization are quite dependent on a number of parameters, including sample preparation and calibration of the analytical tools against appropriate standards. Even if, in a series of measurements, the same method is applied, precise standard operating procedures for sample preparation procedures exist for only a few cases[iii].

As a consequence, the results of different measurement techniques may not be directly comparable. Further, some techniques require samples to be dispersed, the use of suspending agents and/or diluted directly affecting the size measured.

The issue of size is further complicated in that some materials can exist as particles that have external dimensions outside the nanoscale but consist of aggregates or agglomerates of smaller particles or crystals, where these smaller particles are within the nanoscale range. The DIN/ISO proposal describes these as "nano-structured aggregates" or "nano-structured agglomerates." The terms "aggregate" and "agglomerate" are often confused or used interchangeably, but they are in fact quite distinct and are defined as follows:

- Aggregate: particle comprising strongly bonded or fused particles where the resulting external surface area may be significantly smaller than the sum of calculated surface areas of the individual components
- Agglomerate: collection of loosely bound particles or aggregates or mixtures of the two where the resulting external surface area is similar to the sum of the surface areas of the individual components

More specifically, many grades of nanomaterials are manufactured as nanoparticles which are typically 10-20 nm in size at the point of manufacture

[iii] Examples for existing reference nanomaterials are Au nanoparticles from NIST (RM 8011, RM 8012, RM 8013) and silica nanoparticles (ERM-FD100, ERM-FD304) from the European Commission's JRC, which have certified values for their size.

(Fig 1), and some suppliers do quote this as the particle size. However, this can be misleading, as very often these nanoparticles, which are also called "primary particles," form tightly-bound aggregates, which have a somewhat larger size (Fig 1a). When this happens, these aggregates are the smallest particles that actually occur in such a system, for example, a cosmetics product, as the forces required to break apart the aggregates (Fig 1b) are far greater than those usually encountered during production of cosmetic products or application of these products onto skin.

The aggregates may then join together to form more loosely-bound agglomerates (Fig 1c). In the as-supplied dry powder, these agglomerates have particle sizes around 1 micron, placing them well outside the nanoscale range.

In certain cases, individual nanoparticles may form weakly bound agglomerates (Figure 1e), which can separate to nanoparticles by sonication or high shear mixing (Figure 1 f). It is the understanding of the Working Group that this may occur under very dilute conditions or in cases when the attraction forces between particles are weak.

The reported size for any particular sample may differ depending on whether the technique is measuring the size of component nanoparticles, aggregates, and/or agglomerates. Also, any measurement is dependent on how the samples are prepared. This is why the particle sizes can vary so widely; in order to compare results it is necessary to measure them by the same technique, with the samples prepared in the same way.

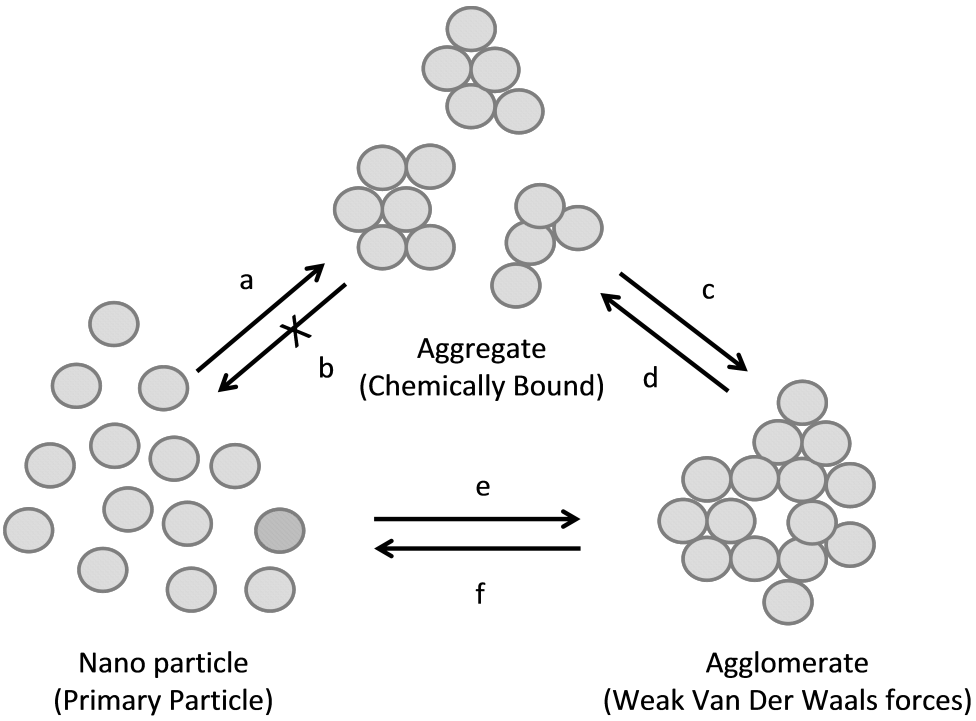

Figure 1. Formation of Aggregates and Agglomerates from Nano Particle Building Blocks.

5.1.1.1.3. Size Range for Nanomaterials

The 2010 Working Group also noted that, as others have reported, there is no particular justification for the 100 nm maximum suggested in numerous other definitions. Many of the properties cited as most significant, such as surface area or number of particles per unit of mass, follow scaling relationships based on classical continuum models throughout the range of interest, while biological activity is not as straightforward or may even be reversed. In some cases, the critical length scale for novel properties and phenomena may be under 1 nm (e.g., manipulation of atoms at ~0.1 nm) or be larger than 100 nm (e.g., nanoparticle reinforced polymers that have the unique feature at ~ 200-300 nm as a function of the local bridges or bonds between the nanomaterials and the polymer). As the size range is not grounded by a chemical or biological underpinning, it is not surprising that different groups have adopted differing ranges. For example, the United Kingdom's Department for Environment, Food and Rural Affairs, in its

Voluntary Reporting Scheme for engineered nanoscale materials, has defined nanoscale materials as having two or more dimensions up to 200 nm, while the Royal Society selected a size range typically from 100 nm down to the atomic level (approximately 0.2 nm).

However, the size range of "approximately 1 nm to 100 nm" is the most commonly used in the various working definitions or descriptions proposed by the regulatory and scientific community. Therefore, in recognition of the value of a uniform, if arbitrary, limit, the 2010 Working Group accepts the more commonly referenced range for nanomaterials, in the realm of 1 to 100 nm.

5.1.1.1.4. In the Final Formulation

The Working Group also added the modifier, "in the final formulation," while recognizing that available methodologies will, in many cases, not allow direct measurements of the particle size in formulated products. Addressing the difficulties of measuring final products has led formulation scientists and regulatory authorities to turn to the raw ingredient suppliers for characterisation information on the powder or simple dispersion systems where a range of techniques are available. Regardless it is important to note the formulation media and processing will affect the nanostructure, and so the nanomaterial the consumer experiences may be quite distinct from the raw material itself.

Therefore, the Working Group felt it important to establish as the goal selection of methods for characterization that, to the extent possible, reflect the size as used, rather than at other life stages (i.e., point of manufacture, as sold, etc.).

5.1.1.2. Criterion: *Stability and persistence of nanomaterials in biological media*

The Working Group believes that nano-ingredients should be sufficiently stable and persistent in biological media to allow for the potential of interaction with biological systems. This would include nano-carriers intended to enhance dermal penetration if they remain stable upon application. Labile nanomaterials, which disintegrate upon

application to skin into their molecular components (e.g., microemulsions, nanoemulsions, or labile liposomes), and subsequently disassociate into their component parts should be excluded. This is consistent with several international bodies, including the European Commission (European Commission 2009), the Scientific Committee on Consumer Safety (SCCS 2007) and the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR 2007). In this context "stable" refers to materials that retain their properties of size and shape in the presence of biological media to allow for the possible interactions with biological systems.

5.1.1.3. **Criterion:** *Solubility*

Additionally, nano-ingredients should be insoluble in water and biological media. For example, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) in its March 2006 report, states that many nanomaterials will have considerable solubility and that for "these materials the interaction with living systems remains close enough to the bulk chemical agent to justify the use of well-established toxicological testing procedures and approaches." This concept is also well recognized by FDA in "Guidance for Industry: Waivers of *In Vivo* Demonstration of Bioequivalence of Animal Drugs in Soluble Powder Oral Dosage Form Products and Type A Medicated Articles (FDA 2008). The conceptual basis cited by FDA for exempting "soluble powders" is that once in solution the product's formulation will usually not influence the bioavailability of the active pharmaceutical ingredient (API).

In this context, "insoluble" refers to a material that retains a non-deformable size and shape when not confined and does not disintegrate in aqueous solution to ionic or molecular forms.

5.2. Characterization of Size

5.2.1. Introduction

The ICCR-5 report on characterisation of nanomaterials concluded, "In particular, methods for characterisation of manufactured nanomaterials in complex matrices such as preparations, final formulations or consumer products, generally are in the stage of development or in the prototypical stage. Hence, if it is deemed important to characterize also a final product (lotion etc.) for its content of manufactured nanomaterials, it is recommended to analyse the state of potentially suitable methods and the associated special needs in a future report."

In the ICCR-5 report, several methods were identified for the measurement of particle size in the realm 1-100 nm and sufficiently mature to be routinely applicable, at least in laboratories with expert knowledge. Those methods in their entirety cover the size range from (below) 1 nm up to and including the micrometer range (Table 1). Of these, only electron microscopy (TEM, SEM), FFF, and AUC cover the entire range of 1 nm – 100 nm as specified in the criteria report, whereas the other methods cover only part of it. A description and discussion of all these methods can be found in the ICCR-5 report.

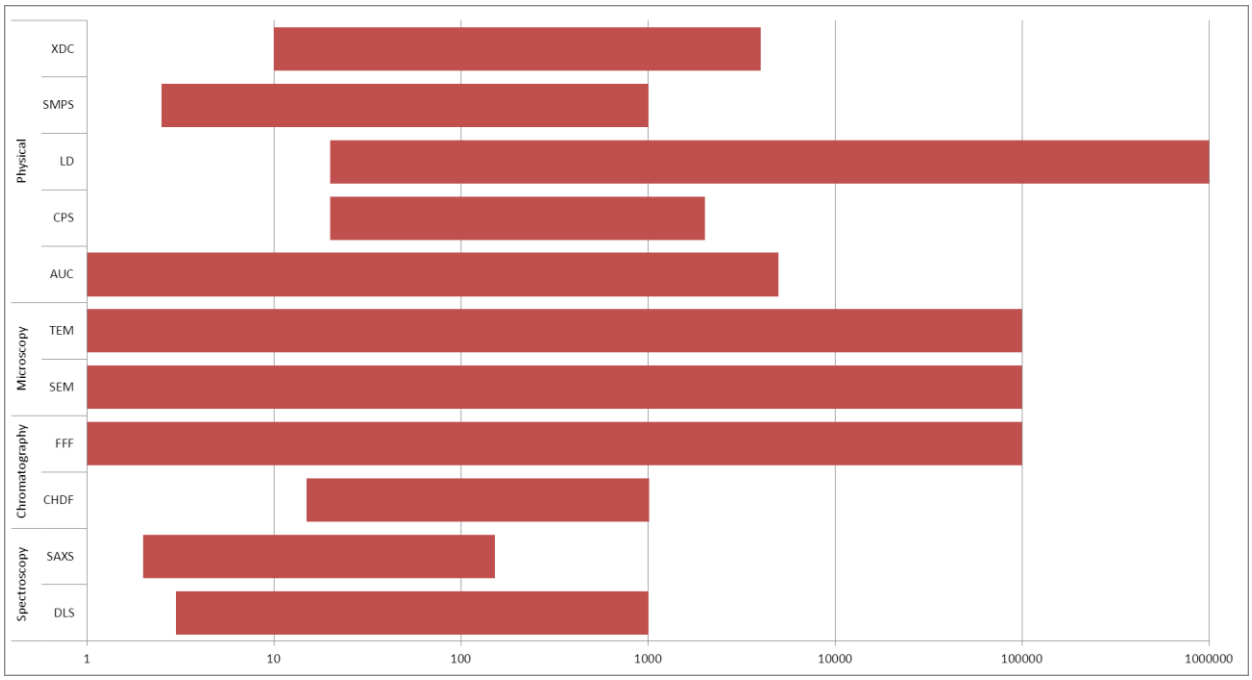

Table 1: Methods for size measurement in the range 1 nm up to more than 100 micrometers.

The characterisation of nanomaterials in complex media, such as cosmetic formulations, generally poses much greater challenges than testing the pristine nanomaterial, because in most cases it is not easy to distinguish the nanomaterial from the surrounding matrix, or because the nanomaterial's properties (e.g., agglomeration state, macromolecule corona, and surface charge) are different from those in its pristine state. The detection and characterisation of nanomaterials in complex media such as food and environmental media has been addressed in several reviews (Hassellov et al. 2008; Tiede et al. 2008; Dudkiewicz et al. 2011), and the need for more research and development in that field is highlighted in those reviews.

If nanomaterials are part of cosmetics products, they come into contact with a vast variety of chemicals and substances. Cosmetics products generally have complex compositions, and the nanomaterial will be only one component. The nanomaterial will interact with the other ingredients of a product, and it will be a challenging task to analyse a certain product for the presence of a nanomaterial. This refers on the one hand to the pure detection of the nanomaterial in a complex chemical formulation that may also contain non-nanosized particles. On the other hand, if quantitative information on nanomaterials in products is also required, it implies not only detection but also the quantification (e.g., the nanoparticle concentration by number, mass, or volume) of the nanomaterial in a certain product.

Systematic methodologies and reliable methods for the detection and characterisation of engineered nanoparticles in complex matrices, including cosmetics formulations, are generally in the stage of development or in the prototypical stage, and there are no national or international standardized methods available for size measurement of nanomaterials in cosmetics formulations. Hence, if it is deemed important to characterize a final or even an intermediate product for its content of manufactured nanomaterials, it is also necessary to analyze the state of potentially suitable methods and eventually promote further development of methods.

Currently there is no method available to detect and characterise a nanomaterial directly in an arbitrary and complex environment. All methods require preparation of the sample before it can be measured. Preparation may include dilution of the analyte or one or more separation steps to extract the nanomaterial to be analysed from a complex formulation. A number of challenges arise that can make the analysis of nanoparticles in complex matrices difficult:

- The separation and/or extraction process itself can change the nanomaterial and may lead to analytical artefacts (aggregation, de-aggregation, etc.).
- There is a lack of SOPs for sample preparation, and therefore difficulties in reproducibility of preparing test items.
- There is a lack of standard and validated methods for analysis of nanomaterials in complex matrices.
- There is a lack of reference or representative test materials.
- There is a distinction between natural and engineered nanoparticles, which is necessary according to the ICCR criteria for nanomaterials and goes along with the monitoring of engineered nanoparticles within a huge background of unintentionally manufactured or natural nanoparticles.
- It may be difficult to decide whether a sample is representative of the entire formulation.

For a more complete discussion of specific methods please see ICCR (2011) "Report of the Joint Regulator - Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials."

5.2.2. Application in Complex Media

Recent reviews have covered the first examples of applications to the analysis of nanoparticles in complex matrices such as food in general (Blasco and Picò, 2011) or were targeted towards the use of specific techniques such as FFF (Kammer et al., 2011),

particle tracking analysis (Gallego-Urrea et al., 2011), and electron microscopy (Dudkiewicz et al., 2011).

FFF – possibly in combination with additional techniques – appears to be promising for the quantitative analysis (size and size distribution) of nanomaterials in final formulations, and first attempts to determine the particle size distribution of titanium dioxide in sunscreen lotions (Contado and Pagnoni, 2008; Samontha et al., 2011) have been published.

Generally, methods of nanomaterials characterisation in complex mixtures, which use a stepwise approach, currently appear more promising than single techniques. The first step of such an approach is often the separation of the nanomaterial from the rest of the sample, for which advanced separation methods are necessary. Suitable methods for the separation step available today include, e.g., FFF (Schimpf 2005) or centrifugation. These two actually refer to a group of methods, e.g., the field used in FFF can be electrical, a transverse flow through a semipermeable membrane, or centrifugal. Centrifugation can be done, e.g., using an Analytical Ultracentrifuge (AU) (Mächtle and Börger 2006) or, more simply, employing the CPS technique (Mächtle and Börger 2006). In any case, the separation step has to be followed by a detection/characterisation step, which can be carried out with a UV-VIS detector, DLS, ICP-MS, Ramandetector or other types of detectors. Examples of coupling separation and detection/analysis of nanomaterials reported in the literature include the combination of FFF and ICP-MS (Dubascoux et al. 2010) or FFF and DLS (Calzolai et al., 2011).

Methods are being developed as well that aim at detecting nanoparticles in (diluted) complex matrices (e.g., environmental, biological, or food samples) without previous separation (Farkas et al. 2011; Gallego-Urrea et al. 2011).

It is well recognized within the general scientific community and noted particularly within various collaborative organizations such as OECD (OECD 2010), that SOPs for

sample preparation and standardised measurement protocols are essential to allow for useful comparison of data. Unfortunately, such SOPs are not yet established for nanomaterials. This is further complicated in that irrespective of the analytical technique used, measurement of nanoparticle size usually requires a simplification of the matrix into which nanoparticles are embedded. To this end, two approaches can be used: either extracting the nanoparticles from the embedding matrix or removing (or at least simplifying) the complex matrix. If any sample preparation treatment is used, it will be necessary to ensure that the treatment method does not modify the original particle size distribution.

As an example of extraction, the soluble substances may be extracted using one solvent or a mixture of solvents according to the section related to solubility (see Section 5.3). The insoluble part can then be analysed, using different methods already described in the previous ICCR recommendations. Nevertheless, it is important to point out that this insoluble part could be made of a large number of substances and that the complete evaluation of the size of the different substances appears very difficult to reach. In this case the insoluble fraction may be further analyzed using a stepwise approach as outlined above. An observation of the insoluble fraction to identify the presence of nanoparticles using electron microscopy is again possible in such a case. Using EDS (or EELS) in combination with electron microscopy, the chemical composition of these nanoparticles may be evaluated.

Alternatively, using TEM, the sample may be dispersed in an appropriate organic solvent and spread on a TEM grid. This will allow the evaluation of the size of the particles present on the grid. The key point of this observation is to get rid of the presence of the organic phase. To complete the observation in the finished product, some particles can be identified using EDS (or EELS) in order to characterize the elemental composition of the particle of interest. It is then possible to discriminate for example between nano or macro titanium oxide.

Another challenge is posed by the need to distinguish between engineered nanoparticles deliberately added to the cosmetics formulation and naturally-occurring nanoparticles. In most cases the techniques used to measure the size of particles cannot provide unambiguous data about their chemical identity. In this case some other techniques will be needed to discriminate between engineered nanoparticles and naturally-occurring nanoparticles.

Based on the current state of the science, direct and routine observation and characterization of nanomaterials in complex media is beyond the capabilities of current methodology. An alternative and useful strategy for characterization of nanomaterials in a finished product could therefore be based on modeling the behaviour of a particular raw material in finished products, until methods for identification and direct characterization of nanomaterials in finished products are available. The characterization of a raw material (ingredient) is expected to be considerably more straightforward, although still much more demanding as compared to the characterization of a pure, monodisperse nanomaterial in a simple dispersing agent (e.g., water). The interaction of such a well-characterized nanomaterial with the main ingredients of the cosmetic product in which it will be used should then be analyzed. This can then give an indication of whether the aggregation/agglomeration state of the nanomaterial in the product will be different as compared to its raw state.

The knowledge of what is considered as a nanomaterial for the chemical and cosmetic industries, and the labeling of the corresponding ingredient in the full ingredient labeling clarify to a great extent what will be present as nano at the final step of the formulation in the products. The observation using the related microscopic techniques will confirm the presence of the substance of interest in relation to the raw material.

5.3. Solubility

For the purposes of this report, the definition and consideration of ‘insolubility’ will be restricted to that provided in ICCR-4 report as described for “Solubility and Stability” in the Discussion:

...nanomaterials should be insoluble in water and biological media. For example, the Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) in its March 2006 report, states that many nanomaterials will have considerable solubility and for "these materials the interaction with living systems remains close enough to the bulk chemical agent to justify the use of well established toxicological testing procedures and approaches." In this context, "insoluble" refers to a material that retains a non-deformable size and shape, when not confined, and does not disintegrate in aqueous solution to ionic or molecular forms.

From this ICCR-4 report excerpt, the major points describing nanomaterials in cosmetics are:

- insoluble in water and biological media
- retains a non-deformable size and shape, when not confined
- does not disintegrate in aqueous solution to ionic or molecular forms

The discussion on insolubility of nanomaterials for this report will be restricted to conditions existing within formulations, and not during or after application of the cosmetic to the human body. Furthermore, the stability and persistence of nanomaterials will be dealt with when discussing the criterion of biopersistence in biological media. It is also recognized that the maintenance of shape and size is dependent on solubility, but it will be discussed in the appropriate section (see Section 5.4).

5.3.1. Clarification of terminology

The term "insolubility" should be distinguished from "immiscibility." Immiscibility is a property of liquids in which they do not mix in any proportion. In contrast, insolubility refers to a solid that does not dissolve in a liquid.

For the purposes of characterization of nanomaterials in cosmetics, a nanomaterial must be insoluble or immiscible in liquid cosmetic formulations. As such, the solid nanomaterial will exist as a colloidal dispersion, with distinct solid and liquid phases. These should be distinguished from suspensions, which refer to coarse particles. Liquid nanoparticles can be described as nanoemulsions, which are liquid dispersions within a liquid (e.g., lipid and water).

5.3.2.Factors affecting solubility

Besides nanomaterial stability or persistence and maintenance of shape and size, the solubility of a material is dependent on the solvent used, temperature, pH, ionic strength of the matrix, and pressure.

When examining nanomaterial solubility in cosmetics, both aqueous and non-aqueous solvents (e.g., organics such as aromatics, alcohols, esters, glycols, and ketones, as well as oil) should be considered, since commercial products are manufactured from both types of matrix. Frequently, a coating (also referred to as a shell or a cap) is employed to alter the solubility of the core nanomaterial. The stability of the nanomaterial with its coating would then be important in this context. Temperature is an important factor affecting solubility. Because only nanomaterials that are insoluble within the formulation will be considered, only temperatures within a narrow ambient range corresponding to cosmetic usage should be considered (i.e., ranging from ~15°C to 30°C). Another factor affecting solubility may be the level of acidity of a commercial product, which is only applicable for charged nanomaterials. As well, pressure may only be important when cosmetic products are formulated as a compressed gas (e.g. hair sprays).

Formulation additives may be added to cosmetics that change the formulations attributes. For example, emulsifiers may be added to increase nanomaterial solubility and dispersability, thickeners such as polymers may be added to alter their consistency, and fragrances, colours, and pH stabilizers may all contribute to how a nanomaterial solubilises within a final product.

5.3.3. Solvents

5.3.3.1. Aqueous solubility

For the purposes of characterization in the context of the ICCR, a nanomaterial in water-based cosmetics must be insoluble or immiscible in aqueous cosmetic formulations. As such, the nanomaterial will exist as a colloidal dispersion, with solid and liquid phases, or a nanoemulsion with two liquid phases (e.g. lipid and water).

The OECD 105 (OECD 1995) Guideline describes methods used for assessing solubility of chemicals. According to the Guideline's definition, the water solubility of a substance is the saturation mass concentration of the substance in water at a given temperature, expressed as the mass of solute per volume of solution (e.g., kg/m3 or g/L). By definition, according to the ICCR criteria, a nanomaterial is an insoluble substance and therefore exceeds its saturation mass concentration in the solute. A preliminary test describes an estimation of solubility based on the appearance of a visual precipitate. If visible solubility is <10-2 g/L, a column elution method is used. If solubility is >10-2 g/L, a flask method is preferred.

5.3.3.2. Non-aqueous solubility

5.3.3.2.1. Fat/Oil Solubility

Cosmetics are frequently formulated in a fat/oil base. Nanomaterials existing in these products must not be miscible in a standard fat matrix. OECD 116 (OECD 1981) defines fat solubility as the mass fraction of a substance which forms a homogeneous phase with a liquid fat (oil) without giving rise to chemical reactions. Again, insoluble material such as nanomaterials would exceed this saturation mass concentration. A standard triglyceride mixture such as HB 307 (NATEC Company, Germany) may be used as the solvent. The method described is only applicable to pure substances that are not reactive with triglycerides. Basically, the material is added to the fat standard until saturation is obtained as measured using a suitable analytical method.

5.3.3.2.2. Alcohol Solubility

Several types of cosmetics are composed of alcohol-based solvents, such as butanol, ethanol, isopropanol, and methanol. A method to determine alcohol solubility is by measuring the partition coefficient of a substance in an alcohol versus water. Usually, a highly non-polar, hydrophobic solvent such as octanol is the standard alcohol used for these tests. The partition coefficient is defined as the ratio of the

equilibrium concentrations of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. The larger the partition coefficient, the greater its solubility in octanol. Therefore, alcohol-insoluble nanomaterials in alcohol-based cosmetics would have a low partition coefficient. This method may also be used to characterise aqueous and lipid (fat/oil) solubility.

5.3.4. Analytical methods to measure nanomaterial solubility

Following the implementation of a given method listed in Section 5.3.3, it would be necessary to quantify the content of the nanomaterial in the aqueous and non-aqueous phases. Several methods have been described in 2011 ICCR report (ICCR 2011) and will not be described in detail here. Depending on the material and the phase examined, almost any method can be employed. In some cases, pre-processing steps may be required, so a combination of methods should also be considered. Furthermore, the most desirable evaluation would involve more than one method for confirmation of the presence, state (e.g. ionic, crystalline), and proportion of nanomaterial. Other important considerations are described below that may be important during study design.

Two general types of strategies could be employed. Either the soluble fraction could be measured and presence of the insoluble nanomaterial could be inferred, or the insoluble nanomaterial could be directly measured. In the former case, presumably the composition of the nanomaterial would be known, and if an element unique to the nanomaterial (i.e., limited concentration in the formulation) is present in relatively high proportions, this element can be used as a tracer for presence of the nanomaterial. A more challenging situation would be where the nanomaterial is present in a very small amount. Then, the method used would need to be sufficiently sensitive to detect the material (See "Report of the Joint Regulator - Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials" (ICCR 2011) for sensitivities of particular methods).

Solubility measurement of any nanomaterial within commercial formulations can be confounded by dilution effects. That is, following dilution with buffered saline for example, the solubility characteristics of the nanomaterial could change. Therefore, the

method(s) selected must examine whole final products at the same concentration as that used in the formulation. The only exception would be in a situation where the raw nanomaterial is available, and it has been empirically demonstrated that the chemico-physical characteristics do not change when diluting the nanomaterial from the commercial product.

5.4. Stability and persistence of nanomaterials in biological media

The ICCR has determined that a nanomaterial must be stable in biological media by demonstrating that it does not change in size or shape. An accurate evaluation of nanomaterial toxicity would be facilitated by assessing the behavior of these materials under conditions of potential exposure.

The Working Group found that no methods have been generally accepted for determining the stability in biological media of nanomaterials used in cosmetic products. However, studies have been published that could provide guidance in the development of this methodology. DMEM and RPMI medium are frequently used media in stability studies (Wiogo, et. al., 2012; Ji, et.al. 2010; Perti-Fink, et.al. 2008). Nanomaterials tend to agglomerate when added to culture media, but the addition of fetal bovine serum or bovine serum albumin (Wiogo, et.al. 2012; Ji, et.al. 2010) to the media has been shown to prevent or reduce agglomeration. The stability of nanomaterials is thereby increased under conditions that more closely simulate in vivo exposure. Initial dispersions of the nanomaterials in media were prepared by sonication (Wiogo, et.al. 2012; Ji, et.al. 2010). Size stability can be assessed by DLS or other appropriate analytical methods and should include a time period in the analysis of size, monitoring the size of a solution of particles in biological media over time to mimic exposure of the product in use will provide information on the persistence of a nanomaterial. The state of dispersion of a nanomaterial may change over the course of application and a substance that may appear to be well dispersed initial could have the opportunity to aggregate and/or agglomerate over a short period of time. Duration of the stability study should therefore simulate exposure to a cosmetic product containing the nanomaterial. Persistence of a nanomaterial in biological fluid is also heavily influenced by shear forces during application of the product to skin and should be considered in the measurement of stability and

persistence. Treating a biological solution containing a nanomaterial with shear before the initial measurement of size, to mimic the forces applied to a product during application, is important. Simple methods such as stirring or sonication before the measurement of size should be considered during development of a protocol.

A factor that may influence solubility in biological media is the concentration of the nanomaterial with higher concentration increasing the tendency for associations between particles. Another factor affecting stability of nanomaterial is the surface charge of the particles (zeta potential). Generally, the greater the zeta potential, the more a dispersion will resist agglomeration or aggregation, and therefore the more stable the nanomaterial.

It is recommended that the solubility and size of the nanomaterial in the cosmetic be known before stability assessment. Then, these same methods could be adapted to give an indication of alterations following addition to biological media. Furthermore, the tendency of a nanomaterial to dissociate in biological media may be measured by examining the ionic speciation of the particle. For example, measurement of the quantity of solubilised ionic zinc in the solubilised fraction of nanodispersion could be used to infer the proportion of intact zinc oxide nanomaterial.

5.5. Standard Reference Materials for Use in the Analysis of Nanomaterials in Cosmetics

Whenever possible and if available, standard reference nanomaterials (SRMs) should be employed to aid in the characterization of nanomaterials within cosmetic formulations. A widely cited definition for SRMs is provided by ISO: “A material that is sufficiently homogeneous and stable, with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process” (ISO Guide 30:1992/Amd 1:2008). Usually, this material has been rigorously characterised for its physical, chemical, and/or biological properties. They can exist in pure form, as standardised solutions/suspensions, and/or within a defined matrix. SRMs may be either non-certified (but tested for homogeneity and stability) or certified (parameters are presented with uncertainty and metrological traceability).

SRMs are important for method validation, calibration of instruments, measurement of uncertainty, personnel training, and quality control and assurance, as well as international and inter-laboratory cooperation[iv]. (European Co-operation for Accreditation 2003)

6. CONCLUSIONS

The Working Group is pleased to submit the report to the ICCR examining methods and strategies to characterize solubility, stability and persistence in biological media, and measurement of size in the realm of 1 to 100 nm in final formulations.

The report provides further clarification and justification for including those criteria. The measurement of solubility was discussed in some detail, with a conclusion that it is important to measure solubility using a wide range of solvents consistent with the wide variety of cosmetic matrices that may be encountered.

However, precise analytical methods and detailed recommendations for the measurement of "stability and persistence in biological media" and "measurement of size in the final formulations" could not be provided. The scientific community is currently starting to address methodologies and strategies for characterization of nanomaterials at the point of use (e.g., stability and persistence in a lotion after application to the skin, or detection and measurement of the size distribution in arbitrarily complex media) and first results were identified. The Working Group believes that while such measurements are the goal, today the scientific and technical state of the art is not yet sufficiently developed to do so.

Therefore in this report the Working Group can recommend the following points be considered:

[iv] Several organizations supply SRMs. Some of these are NIST (nanogold), OECD sponsorship program (nanosilver, nano-Zinc Oxide, CNT), ERM (European Reference Materials; TiO2, ZnO, SiO, CeO2, Ag, MWCNT, nanoclay), LGC Standards (Ag Cerium Oxide, MWCNT, amorphous silica, TiO2, ZnO) Nanocomposix (Au, Ag, TiO2, SiO2, Co, Cu, FeO2, etc.). Additional information may be found at:
NIST Standard Reference Materials: http://www.nist.gov/srm/index.cfm
European Reference Materials: http://www.erm-crm.org/ERM_products/Pages/index.aspx
LGC Standards: http://www.internano.org/content/view/499/251/
Nanocomposix: http://nanocomposix.com/products/silver/reference-materials

- The measured size of a nanoparticle will depend on the methods chosen which may reflect primary particle size, agglomerate size, or aggregated size.
- Many methods require significant sample preparation and so may have little bearing on the nanostructure as used.
- The nature of the formulation will affect the nanostructure.
- Ingredients that are stable in a formulation may not be so in contact with biological media.
- Behavior of nanomaterials in biological media may help to interpret results of toxicological evaluations of ingredients.
- Methods of nanomaterials characterisation in complex mixtures, which use a stepwise approch and involve two or more sequentially applied methods, currently appear more promising than single techniques.
- Models using raw materials to understand the behaviour of nanomaterials in complex media are likely to be available in the short term. Such models can be useful until the technology is sufficiently advanced for direct characterisation of nanomaterials in finished products.
- Experts should exercise great care to avoid having scientifically precise reports that are wholly divorced from the conditions relevant to cosmetic usage.

In conclusion, the identification of nanomaterials for regulatory purposes (i.e., labeling, notifications, etc.) and scientific safety assessment should strive to be accurate and relevant to their usage in cosmetic products.

7 References

- Berne, B.J. and Pecora, R. (2000). Dynamic light scattering: with applications to chemistry, biology, and physics. Dover Publications, Mineola, N.Y., vii: p. 376
- Blasco, C. and Picò, Y., 2011. Determining nanomaterials in food. Trends in Analytical Chemistry (TrAC), **30**(1): 84-99.
- Calzolai, L.; Gilliland, D.; Garcia, C.P.; and Rossi, F. (2011). Separation and characterization of gold nanoparticle mixtures by flow-field-flow fractionation. J Chromatogr A, **1218**(27): 4234-9.
- Carney, R.P. et al. (2011). Determination of nanoparticle size distribution together with density or molecular weight by 2D analytical ultracentrifugation. Nat Commun, **2**: 335.
- Contado, C. and Pagnoni, A. (2008). TiO2 in commercial sunscreen lotion: Flow field-flow fractionation and ICP-AES together for size analysis. Analytical chemistry, **80**(19): 7594-7608.
- Dubascoux, S.; I. Le Hécho; et al. (2010). Field-flow fractionation and inductively coupled plasma mass spectrometer coupling: History, development and applications. Journal of Analytical Atomic Spectrometry, **25**(5): 613.
- Dudkiewicz, A.; K. Tiede; et al. (2011). Characterization of nanomaterials in food by electron microscopy. Trends in Analytical Chemistry (TrAC), **30**(1): 28-43.
- European Chemicals Agency (2008) Guidance for the implementation of REACH: Guidance on information requirements and chemical safety assessment. Chapter R.7a: Endpoint specific guidance.
- Eskandar, N.G.; Simovic, S.; and Prestidge, C.A. (2009). Chemical stability and phase distribution of all-trans-retinol in nanoparticle-coated emulsions. International Journal of Pharmaceutics, **376**: 186–194.
- European Commission (2009) Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, OJ L 342, of 22.12.2009, p. 59, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:EN:PDF
- European Co-operation for Accreditation, The Selection and Use of Standard Reference Materials, Publication Reference EA04/14. June 13, 2003. http://www.european-accreditation.org/n1/doc/ea-4-14.pdf
- Farkas, J., H. Peter, et al. (2011). "Characterization of the effluent from a nanosilver producing washing machine." Environment International **37**(6): 1057-1062.
- FDA (2008) U.S. Department of Health and Human Services, Food and Drug Administration, Center for Veterinary Medicine (CVM) October 6, 2008; Guidance for Industry - Waivers of *In Vivo* Demonstration of Bioequivalence of Animal Drugs in Soluble Powder Oral Dosage Form Products and Type A Medicated Articles.
- FDA (2011), Draft Guidance for Industry, "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology," http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm#_ftn1.
- Gallego-Urrea, J.A.; Tuoriniemi, J.; and Hassellöv, M. (2011). Applications of particle-tracking analysis to the determination of size distributions and concentrations of nanoparticles in environmental, biological and food samples. Trends in Analytical Chemistry (TrAC), **30**(3): 473-483.

- Gun'ko, V.M.; Klyueva, A.V.; Levchuk, Y.N.; and Leboda, R. (2003). Photon correlation spectroscopy investigations of proteins. Adv Colloid Interface Sci, **105**: 201-328.
- Hassellöv, M.; Readman, J.W.; et al. (2008). Nanoparticle analysis and characterization methodologies in environmental risk assessment of engineered nanoparticles. Ecotoxicology **17**(5): 344-361.
- Health Canada Guidance for Industry – Preparation of Veterinary Abbreviated New Drug Submissions – Generic Drugs (Effective 2010/04/01).
- ICCR (2010) Report of the ICCR Joint Ad Hoc Working Group on Nanotechnology in Cosmetic Products: Criteria and Methods of Detection – ICCR-4. Presented to ICCR at the 4th meeting of ICCR (ICCR-4) held July 14, 2010 in Toronto Canada.
- ICCR (2011) Report of the Joint Regulator - Industry Ad Hoc Working Group: Currently Available Methods for Characterization of Nanomaterials. Presented to ICCR at the 5th meeting of ICCR (ICCR-5) held June 29, 2011 in Paris, France.
- ISO 229 WPMN Guidance Document – Nanotechnologies – Guidance on physicochemical characterization manufactured nano-objects submitted for toxicological testing.
- ISPRA (2009) Outcome of the International Workshop on Regulatory Issues regarding the Use of Nanotechnologies in Cosmetics. European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra (Italy), 8-9 July 2009. http://www.fda.gov/InternationalPrograms/HarmonizationInitiatives/ucm193423.htm
- Jensen, K.A.; Kembouche, Y.; et al. (2011). Final protocol for producing suitable manufactured nanomaterial exposure media. Copenhagen, The Nanogenotox Project Consortium.
- Ji, Z.; Jin, X.; George, S; Xia, T.; Meng, H.; Wang, X.; Suarez, E.; Zhang, H.; Hoek, E.M.; Godwin, H.; Nel, A.E.; Zink, J.I. (2010). Dispersion and stability optimization of TiO2 nanoparticles in cell culture media, Environ Sci Technol, **44**: 7309-14.
- Kammer, F.V.D.; Legros, S.; Hofmann, T.; Larsen, E.H.; and Loeschner, K. (2011). Separation and characterization of nanoparticles in complex food and environmental samples by field-flow fractionation. TrAC Trends in Analytical Chemistry, **30**(3): 425-436.
- Kaszuba, M.; Connah, M.T.; McNeil-Watson, F.K.; and Nobbmann, U. (2007). Resolving Concentrated Particle Size Mixtures Using Dynamic Light Scattering. Particle & Particle Systems Characterization, **24**(3): 159-162.
- Laera, S. et al. (2011). Measuring protein structure and stability of protein-nanoparticle systems with synchrotron radiation circular dichroism. Nano Lett, **11**(10): 4480-4.
- Leo, A.; Hansch, C.; and Elkins, D. (1971). Partition coefficients and their uses. Chem Rev **71** (6): 525–616.
- Lorenz, C. et al. (2011). Imaging and characterization of engineered nanoparticles in sunscreens by electron microscopy, under wet and dry conditions. Int J Occup Environ Health, **16**(4): 406-28.
- Mächtle, W. and Börger, L. (2006). Analytical Ultracentrifugation of Polymers and Nanoparticles. Berlin & Heidelberg, Springer Verlag.
- OECD (1995) Guideline for the Testing of Chemicals. OECD 105, Water Solubility.
- OECD (1981) Guideline for the Testing of Chemicals. OECD 116, Fat Solubility of Solid and Liquid Substances.

- OECD (1989) Guideline for the Testing of Chemicals. OECD 117, Partition Coefficient (n-octanol/water), High Performance Liquid Chromatography (HPLC) Method.
- OECD (2009) Preliminary Review of OECD Test Guidelines for their Applicability to Manufactured Nanomaterials. ENV/JM/MONO(2009)21. Paris 2009.
- OECD (2010). Preliminary guidance notes on sample preparation and dosimetry for the safety testing of manufactured nanomaterials.
- Petri-Fink, A.; Steitz, B.; Finka, A.; Salaklang, J.; Hofmann, H. (2008) Effect of cell media on polymer coated superparamagnetic iron oxide nanoparticles (SPIONs): colloidal stability, cytotoxicity, and cellular uptake studies, Eur J Pharm Biopharm, 68, 129-37.
- Reed, R.B. et al. (2012) Environmental Toxicology and Chemistry **31**(1):93-99.
- Samontha, A.; Shiowatana, J.; and Siripinyanond, A. (2011). Particle size characterization of titanium dioxide in sunscreen products using sedimentation field-flow fractionation-inductively coupled plasma-mass spectrometry. Analytical and Bioanalytical Chemistry **399** (2): 973-978.
- SCCP (2007) Scientific Committee on Consumer Products: Opinion on Safety of Nanomaterials in Cosmetic Products. Adopted by the SCCP after the public consultation on the 14th plenary of 18 December 2007.
- SCENIHR (2007) Scientific Committee on Emerging and Newly-Identified Health Risks Opinion on the appropriateness of the risk assessment methodology in accordance with the technical guidance documents for new and existing substances for assessing the risks of nanomaterials, adopted this opinion at the 19th plenary on 21-22 June 2007.
- Schimpf, M.E. (2005). Field-Flow Fractionation. Analytical Instrumentation Handbook. J. Cazes. New York, Marcel Dekker.
- Tiede, K.; Boxall, A.B.A.; et al. (2008). Detection and characterization of engineered nanoparticles in food and the environment. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment **25**(7): 795-821.
- Tiede, K.; Tear, S.P.; David, H.; and Boxall, A.B. (2009). Imaging of engineered nanoparticles and their aggregates under fully liquid conditions in environmental matrices. Water Res, **43**(13): 3335-43.
- Wiogo, H.T.; Lim, M.; Bulmus, V.; Guitierrez, L.; Woodward, R.C.; Amal, R. (2012). Insight into serum protein interactions with functionalized magnetic nanoparticles in biological media. Langmuir, **28**, 4346-56.

APPENDICES

Appendix 1: Summary of selected definitions for Nano Technology, Nanoscale, and Nanoparticles

Health Canada[i]	Health Canada considers any manufactured substance or product and any component material, ingredient, device, or structure to be nanomaterial if: a. It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale, or; b. It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena. For the purposes of this definition: i. The term "nanoscale" means 1 to 100 nanometres, inclusive; ii. The term "nanoscale properties/phenomena" means properties which are attributable to size and their effects; these properties are distinguishable from the chemical or physical properties of individual atoms, individual molecules and bulk material; and, iii. The term "manufactured" includes engineering processes and the control of matter.
EU Cosmetics Regulation (Article 2 Definitions (k))[ii] – also see the provision[iii]*	*"Nanomaterial"* means an insoluble or biopersistant and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.
COMMISSION RECOMMEND-ATION (2011/696/EU) of 18 October 2011 on the definition of nanomaterial[iv]	"Nanomaterial" means a natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1 nm - 100 nm.
EU Regulation 1169/2011 on the provision of food information to consumers (Article 2 Definitions (2) (t)[v]	"Engineered nanomaterial" means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic to the nanoscale.

EU Novel food Regulation (Article 3) Definitions (2)(f)[vi]	"Engineered nanomaterial" means any intentionally produced material that has one or more dimensions of the order of 100 nm or less or is composed of discrete functional parts, either internally or at the surface, many of which have one or more dimensions of the order of 100 nm or less, including structures, agglomerates or aggregates, which may have a size above the order of 100 nm but retain properties that are characteristic to the nanoscale.
European Medicines Agency (EMEA)[vii]	From the atomic level at around 0.2 nm (2 Å) up to around 100 nm.
FDA[viii]	When considering whether an FDA-regulated product contains nanomaterials or otherwise involves the application of nanotechnology, FDA will ask: 1. Whether an engineered material or end product has at least one dimension in the nanoscale range (approximately 1 nm to 100 nm); or 2. Whether an engineered material or end product exhibits properties or phenomena, including physical or chemical properties or biological effects, that are attributable to its dimension(s), even if these dimensions fall outside the nanoscale range, up to one micrometer.
NNI[ix]	Nanotechnology is the understanding and control of matter at dimensions between approximately 1 and 100 nm, where unique phenomena enable novel applications….Dimensions between approximately 1 and 100 nm are known as the nanoscale. Unusual physical, chemical, and biological properties can emerge in materials at the nanoscale. These properties may differ in important ways from the properties of bulk materials and single atoms or molecules.
National Organic Standards Board[x]	Engineered nanomaterials: substances deliberately designed, engineered and produced by human activity to be in the nanoscale range (approx 1-300 nm) because of very specific properties or compositions (e.g. shape, surface properties, or chemistry) that result only in that nanoscale. Incidental particles in the nanoscale range created during traditional food processing such as homogenization, milling, churning, and freezing, and naturally occurring particles in the nanoscale range are not intended to be included in this definition. All nanomaterials (without exception) containing capping reagents or other synthetic components are intended to be included in this definition.
OECD[xi]	Nanotechnology is the set of technologies that enables the manipulation, study or exploitation of very small (typically less than 100 nanometres) structures and systems.
SCCP[xii]	A nanoparticle is a particle with one or more dimensions at the nanoscale (at least one dimension <100nm). A nanomaterial is a material with one or more external dimensions, or an internal structure, on the nanoscale, which could exhibit novel characteristics compared to the same material without nanoscale features. Nanoparticles can be divided into two groups: i) soluble and/or biodegradable nanoparticles which disintegrate upon application to skin into

	their molecular components (e.g. liposomes, microemulsions, nanoemulsions), and ii) insoluble particles (e.g. TiO2, fullerenes, quantum dots).
US Nanoscale Science, Engineering and Technology (NSET) Subcommittee of the National Science and Technology Council's Committee on Technology (February 2000)[xiii]	The length scale of approximately 1-100 nanometer range. […] In some particular cases, the critical length scale for novel properties and phenomena may be under 1 nm (e.g., manipulation of atoms at ~0.1 nm) or be larger than 100 nm (e.g., nanoparticle reinforced polymers have the unique feature at ~ 200-300 nm as a function of the local bridges or bonds between the nano particles and the polymer).
UK Royal Society & Royal Academy of Engineering[xiv]	Typically from 100 nm down to the atomic level (approximately 0.2 nm).

[i] http://www.hc-sc.gc.ca/sr-sr/pubs/nano/pol-eng.php

[ii] EU Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products, OJ L 342, of 22.12.2009, p. 59, http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2009:342:0059:0209:EN:PDF Last checked April 15, 2012.

[iii] *Article 2 Paragraph 3: "in view of the various definitions of nanomaterials published by different bodies and the constant technical and scientific developments in the field of nanotechnologies, the Commission shall adjust and adapt point (k) of paragraph 1 to technical and scientific progress and to definitions subsequently agreed at the international level."

[iv] http://ec.europa.eu/environment/chemicals/nanotech/index.htm

[v] Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, and repealing Commission Directive 87/250/EEC, Council Directive 90/496/EEC, Commission Directive 1999/10/EC, Directive 2000/13/EC of the European Parliament and of the Council, Commission Directives 2002/67/EC and 2008/5/EC and Commission Regulation (EC) No 608/2004; http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:304:0018:0063:EN:PDF

[vi] Position of the European Parliament adopted at first reading on 25 March 2009 with a view to the adoption of Regulation (EC) No .../2009 of the European Parliament and of the Council on novel foods, amending Regulation (EC) No 1331/2008 and repealing Regulation (EC) No 258/97 http://www.europarl.europa.eu/sides/getDoc.do?pubRef=-//EP//TEXT+TA+P6-TA-2009-0171+0+DOC+XML+V0//EN&language=EN)

[vii] European Medicines Agency. Reflection Paper on Nanotechnology-Based Medicinal Products for Human Use. EMEA/CHMP/79769/2006, p. 3 (2006); www.emea.europa.eu/pdfs/human/genetherapy/7976906en.pdf.

[viii] Draft Guidance for Industry, "Considering Whether an FDA-Regulated Product Involves the Application of Nanotechnology", http://www.fda.gov/RegulatoryInformation/Guidances/ucm257698.htm#_ftn1

[ix] http://www.nano.gov/NNI_Strategic_Plan_2007.pdf

[x] National Organic Standards Board Meeting, Madison, Wisconsin, October 25 – 28, 2010 http://www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELPRDC5086584

[xi] http://www.oecd.org/sti/nano

[xii] Scientific Committee on Consumer Products, Opinion on Safety of Nanomaterials in Cosmetic Products, Adopted 18 December 2007, http://ec.europa.eu/health/ph_risk/committees/04_sccp/docs/sccp_o_123.pdf

[xiii] www.nsf.gov/crssprgm/nano/reports/omb_nifty50.jsp

[xiv] The Royal Society and the Royal Academy of Engineering, "Nanoscience and nanotechnologies". Chapter 2 "What are nanoscience and nanotechnologies?", 2004; http://www.nanotec.org.uk/finalReport.htm